DISCUSSION
Toxic heavy metals are widely found in human environment due to the anthropogenic activities. These metals act as catalysts in the oxidative reactions of biological macromolecules, therefore, it is plausible that the toxicities associated with these metals might be due to oxidative tissue damage. Arsenic is a naturally occurring ubiquitous element. Exposure to high levels of arsenic has been recognized for many decades in some regions of the world. Millions of people are at risk of cancer and other diseases because of chronic arsenic exposure. Studies on this metal are carried out for hazard identification and safety assessment to protect the health of exposed life. Since not much information is available on endocrine organs, long-term (chronic) effect of trivalent arsenic has been investigated in ovary, adrenal, pancreas and thyroid gland of the adult female mice. The therapeutic effects of AP and MLT were also explored in view of the widespread arsenic induced health hazards the world over.

The in vivo studies on effects of arsenic trioxide (As$_2$O$_3$) was carried out in some endocrine organs (ovary, adrenal, pancreas and thyroid gland) of adult female mice (Mus musculus) followed by ameliorative effect of Andrographis paniculata (AP) compound and melatonin (MLT) on arsenic induced toxicity in a mouse model. The selected doses of arsenic trioxide (As$_2$O$_3$) in the present study were (0.5 and 1.0 mg/kg body weight, po) based on the LD$_{50}$ of arsenic trioxide while that of AP (50 mg/kg body weight, po) and MLT (10 mg/kg body weight, i.p) were based on the earlier publications.
(Singh et al., 2001; Sokkary et al., 1999) and again standardized in the laboratory. The treatment of toxicant (As$_2$O$_3$) and *Andrographis paniculata* (AP) were administered orally using a feeding tube attached to a hypodermic syringe while antioxidant melatonin was injected intraperitoneally 25-30 minutes before arsenic feeding due to rapidity of melatonin metabolism (Vakkuri, 1985). Oral mode of administration was selected since drinking water is the major source of arsenic contamination in the world. The duration of the treatment was of 30 days. Various parameters studied at the end of treatment were body and organ weights, metabolic and antioxidant parameters, histological analysis and arsenic retention. Levels of total protein were estimated in all endocrine organs (ovary, adrenal, pancreas and thyroid gland) to find out the effect of As$_2$O$_3$ on the protein metabolism. In addition, some specific parameters in steroidogenic organs (ovary and adrenal) viz., activities of 3β and 17β hydroxysteroid dehydrogenase (HSDs) and cholesterol levels were investigated to study the alterations in steroidogenesis. To evaluate free radical induced cell injury by As$_2$O$_3$, the activities of some antioxidant enzymes, viz., glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and levels of lipid peroxides (LPO), glutathione (GSH), ascorbic acid (AA) were determined in all the above mentioned endocrine organs. In order to investigate the effect of arsenic on pancreas, some specific parameters viz., activities of serum amylase, serum lipase, levels of total sulphydryl groups and blood glucose were investigated to study the alteration in exocrine and endocrine regions of pancreas. Levels of serum protein and cholesterol were too determined.
Organ weight changes have long been accepted as a sensitive indicator of chemically induced changes to organs. In toxicological experiments, comparison of organ weights between treated and untreated groups of animals have conventionally been used to predict the toxic effect of the test article (Peters and Boyd, 1966; Pfeiffer, 1968). In arsenic treated groups of our study, body and organ weights were significantly decreased with respect to control indicating the systemic toxicity. These reduced gravimetric values thus indicated the lethal nature of the toxicant. Ovarian weights were considered valuable in sexually mature animals because they could indicate ovarian dysfunction. Pancreas, adrenal and thyroid gland weights in addition body weights were also considered to be a sensitive predictor of toxicity and correlated well with histopathological findings. Corroborating with the present study Avani and Rao (2006) reported altered gravimetric data in arsenic treated animals. Similarly, several authors also found a reduction in body and organ weights after As$^{3+}$ treatment (Jhala and Chinoy, 2004; Chattopadhyay et al., 2002).

The results of the present study demonstrated a significant decline in protein levels in all organs and in serum (Biswas et al., 2000) following arsenic exposure, with a marked decrease in the high dose groups. Reduction in protein levels in them could be attributed to their damage by singlet oxygen, often due to oxidation of essential amino acids, viz., methionine, tryptophan, histidine or cysteine residues (Halliwell and Gutteridge, 1985). Arsenite (As$^{3+}$) having a high affinity for thiol groups in proteins, can form complexes with
vicinal thiols and sulfhydryl groups containing proteins. Hence, it inhibits more than 200 enzymes (Klassen et al., 1986; Robert and Judd, 1986; Lagerkrist and Zetterlund, 1994; Aposhian, 1997; Roy and Saha, 2002; Jhala and Chinoy, 2004).

Since, the production of reactive oxygen species is high in reproductive tissue due to active metabolism and steroidogenesis, the tissue is under continuous stress. An increase in ovarian cholesterol levels in the present investigation by arsenic treatment suggested alteration in its utilization, probably due to a simultaneous decline in the activities of 3β and 17β-HSDs which would affect ovarian steroidogenesis. Similarly a reported decrease in the activities of 3β and 17β HSDs along with elevated cholesterol level in adrenal gland of mice treated with arsenic implies that in arsenic intoxication, adrenal steroidogenesis would also be impaired. In steroidogenesis, 3β hydroxysteroid dehydrogenase (3β HSD) and 17β hydroxysteroid dehydrogenase (17β HSD) are the key regulatory enzymes. A decrease in the plasma levels of estradiol in arsenic-treated animals occurred due to the inhibition of ovarian steroidogenic enzyme activities, because these are responsible for the regulation of ovarian estradiol synthesis (Hinshelwood et al., 1994). Similar findings were observed by Chattopadhyay et al (1999, 2003) who noted weight loss of the female sex organs may be due to the possibility of low plasma levels of gonadotropins and estradiol. Sarkar et al (1991) and Chinoy (2002, 2004) also reported that suppressed activities of these enzymes by arsenic treatment associated with inhibition in testicular and ovarian steroidogenesis. In histological findings of ovary after treatment of arsenic revealed structural alterations, necrosis, follicular atresia, dense
vacuolization and decreased diameter and number of follicles in the stromal tissue. The histopathological analysis of adrenal gland too showed zonal irregularity and substantial hypertrophy of cellular medullary elements. Zhang and Tan (2005) indicated extents of histopathological changes developed by arsenic on reproductive and endocrine system of female rats which also supported this result.

Ascorbic acid has long been known as a powerful antistress and detoxifying agent with antioxidant and detoxifying properties, which could scavenge free radicals formed in the system (Kutsky, 1973; Chinoy, 1978; Basu and Dickerson, 1996). A decrease in level of total ascorbic acid in steroidogenic organs (ovary and adrenal) in the present study, indicated arsenic induced stress leading to rapid utilization of stored ascorbic acid or else non-conversion of dehydro ascorbate (DHA) to reduced ascorbic acid due to a reduction in GSH. Alteration in ascorbic acid metabolism has also been reported in several organs of arsenic treated rats and mice (Chinoy, 2002; Avani and Rao, 2006).

The mechanism of arsenic toxicity to individual cell type has historically centered around the inhibitory effects on cellular respiration at the level of mitochondria. Disruption of oxidative phosphorylation and concomitant decrease in the cellular levels of ATP (Chen et al., 1986) are thought to be important central events of arsenic-induced toxicity evoking increased production of hydrogen peroxide. These effects could cause formation of reactive oxygen species (ROS) resulting in oxidative stress (NRC, 1999). The reactive oxygen species (ROS) generated by arsenic react with polyunsaturated fatty acid and cause peroxidative changes that result in
enhanced lipid peroxidation (Ramos et al., 1995). Lipid peroxidation is regarded as one of the basic mechanisms of tissue damage caused by free radicals (Esterbauer et al., 1991). In our study, lipid peroxidation levels significantly increased in endocrine organs of arsenic treated animals, thus corroborating well with the earlier speculation that arsenic induces oxygen free radicals or promotes formation of lipid peroxides (Borcea et al., 1999; Ramos et al., 1995; Rao and Avani, 2004), and high levels of such lipid peroxidation products have been indicated in the development of diabetes (Lyons, 1991; Sinclair, 1993).

Superoxide dismutase and catalase are considered primary enzymes since these are involved in the direct elimination of ROS (Halliwell and Gutteridge, 1985). SOD is an important defence enzyme, which catalyzes the dismutation of superoxide radicals ($O_2^-$ to $H_2O_2$) (Nissen and Kreysel, 1983). Catalase is a heme protein, which catalyzes the reduction of hydrogen peroxides (converts $H_2O_2$ to oxygen and water) and protects the tissues from highly reactive hydroxyl radicals (Chopra et al., 1958). Therefore, reduction in the activities of SOD and catalase caused deleterious effects in above mentioned organs of arsenic exposed mice which could be due to the accumulation of superoxide anion radicals and hydrogen peroxide. Glutathione peroxidase (GPx), a Se-containing enzyme detoxifies $H_2O_2$ to $H_2O$ through the oxidation of GSH. Depression of GPx activity was observed in endocrine organs of arsenic-exposed animals indicates the reduction in the levels of GSH and increase in the levels of peroxides. Thus, inhibition of antioxidant enzymes viz., SOD, catalase and GPx in arsenic-exposed animals also point to a disturb antioxidant defense system of cells which might lead to
generation of oxidative stress. Wu et al (2001) suggested that ingestion of arsenic contaminated well water may cause deleterious effects by increasing the level of reactive oxidants and decreasing the levels of antioxidant capacity in plasma of individuals.

GSH is a critical component of the oxidant defense system which helps in scavenging free radicals generated during arsenic poisoning (Hwang et al., 1993). GSH play significant role in the reduction of As(V) to As(III) in blood and in the methylation reaction of inorganic arsenic in liver (Pi et al., 2002). It protects the membrane polyunsaturated fatty acids from peroxidation and has an antioxidant function. In the present study, exposure to As led to a significant depletion of GSH in the endocrine organs. This reduction is suggested to be due to the consumption of glutathione while protecting against the arsenic induced oxidative stress, as it helps to maintain cellular redox status, and plays an important role in protecting against arsenic toxicity (Hughes, 2000). Acute administration of arsenic to rats produced a significant reduction in hepatic GSH (Maiti and Chatterjee, 2001). Similarly, chronic exposure of arsenic to rats and mice via injections caused upto 35% depletion in hepatic GSH, along with liver injury (Flora, 1999; Liu et al., 2000).

Acute arsenite toxicity, including its effects on glucose metabolism, is generally attributed to its reactivity towards thiol (SH) groups (Aposhian, 1989; NRC 1999). Sulphydryl groups are prone to be damaged by free radicals resulting in their oxidation (Halliwell and Gutteridge, 1985). A number of sulfhydryl containing proteins and enzyme systems have been found to be altered by exposure to arsenic (Robert and Judd, 1986; Roy and Saha, 2002). A steep decline in the levels of total thiol (sulphydryl) groups was noted in
pancreas after arsenic exposure. Studies by Halliwel and Gutteridge (1985) also show decreased levels of protein by arsenic intoxication in support of our data. Several previous reports confirm these findings (Rao and Avani, 2004; Ramanathan, 2002). Trivalent arsenic reacts in vitro with sulphydryl groups of glutathione, haemoglobin, proteins, amino acids or an enzyme forming As-SH complexes (Pi et al., 2002; Delnomdedieu et al., 1994; Lagerkvist and Zetterlund, 1994) and this property of arsenic is generally considered to be its mechanism of action by which it exerts toxicity.

The present study also revealed that exposure of arsenic possibly induces diabetes mellitus in the mice, manifested by alteration of normal blood glucose level (Biswas et al., 2000). These changes thus corroborated well with the findings of earlier survey reports that arsenic might induce diabetes mellitus (Chen et al., 1992; Lai et al., 1994; Rahman and Axelson, 1995; Rahman et al., 1995, 1998; Tsai et al., 1999; Tseng et al., 2000). The potential biological mechanisms of arsenic-induced diabetes based on the current knowledge of the biochemical properties of arsenic. Arsenate can substitute phosphate in the formation of adenosine triphosphate (ATP) and other phosphate intermediates involved in glucose metabolism, which could theoretically slow down the normal metabolism of glucose, interrupt the production of energy, and interfere with the ATP-dependent insulin secretion. On the other hand, arsenite has high affinity for sulfhydryl groups and thus can form covalent bonds with the disulfide bridge in the molecules of insulin, insulin receptors, glucose transporters (GLUTs), and enzymes involved in glucose metabolism. As a result, the normal functions of these molecules can be hampered. Induction of oxidative stress as indicated by alterations in
antioxidant indices viz. SOD, GPx, GSH, LPO etc. and interference in signal transduction or gene expression by arsenic or by its methylated metabolites are the most possible causes to arsenic-induced diabetes mellitus through mechanisms of induction of insulin resistance and beta cell dysfunction (Tseng, 2004). ROS can function as signaling molecules to activate a number of cellular stress-sensitive pathways linked to insulin resistance and decreased insulin secretion (Izquierdi-Vega et al., 2006). A relationship in between arsenic induced oxidative stress in pancreas (Izquierdo et al., 2006; Mukherjee et al., 2006) and development of diabetes mellitus has also been proposed by many researchers (Borcea et al, 1999; Diaz-Villasenor et al., 2006).

Serum amylase and lipase are enzymes found primarily in the pancreas. The primary importance of measuring these enzymes is to check the pathological condition of the pancreas. A markedly increased amylase and lipase activities in pancreas was the indication of extensive tissue and cellular damage in test animals. In support to our data, studies by Hotaling (1998) reported that arsenic administration elevated the serum level of these enzymes due to impairment of acinar tissues. In histological findings, pancreatic tissue displayed sign of inflammation, tissue damage, vacuolization, damage of acini, rupture of islet cell, shrinkage and reduced number of islet cell. Chronic oral exposure to arsenic in situ caused significant decrease in population of pancreatic islet cells (Mukherjee et al., 2003, 2006). Thus, histopathological changes coincide with the altered biochemical studies, which indicate extent of toxicity developed by arsenic on pancreas.
Free radicals and reactive oxygen species (ROS) participate in physiological and pathological processes in the thyroid gland. Thyroid epithelial cells produce moderate amounts of reactive oxygen species (ROS) that are physiologically required for thyroid hormone synthesis. They are not necessarily toxic because they are continuously detoxified either in the process of hormone synthesis or by endogenous antioxidant system. However, when they are too much produced, they may become toxic. It is therefore crucial for thyrocytes to be efficiently protected against excessive ROS production. Thus, to face the oxidative challenge and survival, thyrocytes have developed protective systems that limit the toxicity of endogenously and naturally produced ROS. They include antioxidant enzymes such as superoxide dismutases, catalase, glutathione peroxidase, and peroxiredoxins (Mutaku et al., 2002; Mano et al., 1997; Gerard et al., 2005; Kim et al., 2000). In present study, arsenic induced oxidative stress observed in thyroid gland of treated animals as revealed by increase in lipid peroxidation and changes in antioxidative indices (SOD, CAT, GPx, GSH) indicated extent of arsenic toxicity on thyroid gland of mice. In histological analysis, thyroid tissue showed several microfollicles, interfollicular edema, reduced colloid content of the follicles, and hypertrophy of glandular epithelial cells. This result was also supported by Glattre et al., (1995) and Biswas et al., (2000) which indicate extent of toxicity developed by arsenic on rat thyroid.

Arsenic accumulation in the endocrine (Glattre et al., 1995) and various other organs further supported arsenic exerted toxic effects by affecting their structural, biochemical and functional aspects, in support of earlier published
data (Rao and Avani, 2004; Nandi et al., 2005; Biswas et al., 2000). The above data clearly elucidate alterations in endocrine organ structure which would influence their functions. Thus, our findings suggested that arsenic feeding to mice induced toxic effects in ovary, adrenal, pancreas and thyroid glands.

**ROLE OF ANDROGRAPHIS PANICULATA /KALMEGH**

In the present study, when *Andrographis paniculata* (AP) is supplemented along with high dose of arsenic to mice, a remarkable resurgence was observed in the body and organ weights, antioxidant enzymes- SOD, GPx and catalase, together with the levels of glutathione, lipid peroxidation, and ascorbic acid. Levels of protein, total –SH groups, blood glucose, serum protein, serum cholesterol and activities of serum amylase and lipase along with arsenic retention in the AP supplemented groups were comparable to control value. In the existing study, the enzymes of steroidogenesis viz., 3β hydroxysteroid dehydrogenase and 17β hydroxysteroid dehydrogenase along with cholesterol level were found to recover from the arsenic induced toxicity. Further, histology of ovary with healing in the stromal tissue and the follicles found to be normal with no vacuolization. A pronounced recovery in the thickness of the cortex was seen and normal medullary elements of adrenal glad were observed. A significant recovery in the morphology and population of cells in pancreas were observed. In section of AP treated thyroid gland, appeared normal follicles, colloid and interfoliicular area. Thus, biochemical and histopathologic score showed alleviated pathological damage in above mentioned endocrine organs.
after being treated with *Androgaphis paniculata*, which suggested the protective role of AP of herbal origin.

The protective role may be due to the direct reaction of compounds of *Andrographis* with free radicals (Chiou et al., 2000; Trivedi and Rawal, 2001). Andrographolide has been reported to be efficient at regulating immune responses (Calabrese et al., 2000; Rajagopal et al., 2003). This molecule has recently been shown to work as an anti-inflammatory agent by reducing the generation of reactive oxygen species in human neutrophils (Shen et al., 2002). In the current study, significant decrease in LPO levels and significant increase in GSH level in the endocrine organs of AP supplemented animals, suggested accelerating the repair mechanism of damaged cell membranes. These certainly indicate that AP contains active substances, which are capable of preventing LPO, a natural deleterious process. Increased activities of GPx and catalase in AP supplemented animals suggested that the antioxidant effects elucidated by AP are possible due to their ability to activate antioxidant enzymes that catalyze the reaction of oxidants. Studies by Sheeja et al., (2006) reported that AP inhibits formation of oxygen derived free radicals and normalized the level of antioxidant indices. The enzyme superoxide dismutase and glutathione constitute the first line of defense against free radical induced damage and a restoration of the SOD activity and GSH, ascorbic acid and total –SH groups levels by AP may account for its protective effect against free radicals and thus maintain low level of lipid peroxides (Zhang and Tan, 2000b; Verma and Vinayak, 2007; Shen et al., 2000).
In a pharmacological study, Kapil et al., (1993, 1994) also demonstrated that andrographolide protected rat liver against hepatotoxins carbon tetrachloride (CCL\textsubscript{4}) and tert-butyl hydroperoxide (tBHP) by reducing the levels of lipid peroxidation product malondialdehyde (MDA) and by maintaining high levels of the reduced form glutathione (GSH). They suggested that inhibition of malondialdehyde formation revealed the free radical scavenging properties of diterpene lactone andrographolides. AP has been demonstrated a number of different pharmacological actions in vitro and animal studies. Recently AP extracts shown to have antitumoral (Rajagopal et al., 2003), anti-inflammatory (Gabrielian et al., 2002), cardiovascular (Thisoda et al., 2006) and antiviral properties (Calabrese et al., 2000). In vitro use of Andrographis paniculata along with arsenic brought about substantial decrease in the genotoxic endpoints (Avani and Rao, 2006). Findings by Sing et al., (2001), Trivedi et al., (2001) and Wang et al., (1997) have also suggested an increase in the cellular non protein thiol levels in vivo as well as the antioxidant potential of this plant on Swiss albino mice and rabbits.

Oxidative stress is believed to be a pathogenetic factor in the development of diabetic complications. The present study also revealed that exposure of arsenic possibly induces diabetes in female mice manifested by alteration of normal blood glucose level. These changes thus corroborated with the findings of earlier survey reports that arsenic might induce diabetes mellitus (Tseng et al., 2000; Rahman et al., 1998). In previous studies aqueous extract of AP (Husen et al., 2004), ethanolic extract of AP whole plant (Zhang and Tan, 2002), ethanolic extract of the aerial parts of AP (Zhang and Tan, 2002) had shown significant antihyperglycemic activities.
against streptozotocin induced diabetic rats. Andrographolide, an active principle in the leaves of AP has been shown to have significant antidiabetic activity (Yu et al., 2003) and suggested that andrographolide can increase glucose utilization to lower plasma glucose in diabetic rats lacking insulin. The mechanism of action of Andrographis is by increasing peripheral utilization of glucose, probable by potentiating insulin action and not by a direct insulin releasing action on islet cells in pancreas. Thus, AP had reputable use in treating diabetes (Ahmed and Asmawi, 1993; Husen et al., 2004).

Pharmacological studies have demonstrated that andrographolide have improved antioxidant status of intoxicated and diabetic animals, suggesting that they functioned as chain-breaking antioxidants. Though exact mechanism of action of andrographis is not known, however, based on the above reports it is plausible that scavenging of free radicals and hydrogen transfer by andrographolide might play an important role in providing protection against arsenic induced oxidative damage in our study.

PART – II : EFFECTS OF ARSENIC AND ROLE OF MELATONIN (MLT)

The results of the present study demonstrated significant decline in protein levels in all organs and in serum (Biswas et al., 2000) following arsenic exposure, with a marked decrease in the high dose groups. Serves et al, (1995) also reported that interactions between As(III) and thiol containing proteins and peptides have generally been regarded as a basis for the effects of arsenic on the structure and function of these molecules. Decline in protein levels might be related to the possible inhibition of DNA synthesis by arsenic. Lack of adequate protein turnover would have an adverse effect on the available enzymes, receptors and structural proteins.
The increase in ovarian and adrenal cholesterol levels in the present investigation by arsenic treatment suggested alteration in its metabolism, probably due to a simultaneous decline in the activities of 3β and 17β-HSDs which would affect ovarian and adrenal steroidogenesis. Alterations in the activities of these two enzymes affects hormone production (Jana et al., 2006) and hence reduced gravimetric data and total protein levels of treated animals occurred in our study. Similar findings were observed by Chattopadhyay et al (1999, 2003) who noted weigh loss of the ovary may be due to the possibility of low plasma levels of gonadotropins and estradiol. Sarkar et al (1991) and Chinoy et al (2002, 2004) also reported that suppressed activities of these enzymes by arsenic-treatment associated with inhibition in testicular and ovarian steroidogenesis. In histological findings of ovary after treatment of arsenic revealed structural alterations, necrosis, follicular atresia, dense vacuolization and decreased diameter and number of follicles in the stromal tissue. The histopathological analysis of adrenal gland too showed zonal irregularity and substanstial hypertrophy of medular elements. Zhang and Tan (2005) also showed histopathological changes developed by arsenic on reproductive and endocrine system of female rats to support our data.

Ascorbic acid is known to be a powerful reducing agent, which helps in activating several enzymes, and act as an antioxidant for detoxifying toxic substances (Kutsky, 1973). A decrease in level of total ascorbic acid in steroidogenic organs (ovary and adrenal) in the present study, indicating arsenic induced stress leading to rapid utilization of stored ascorbic acid or else non-conversion of DHA to reduced ascorbic acid due to decrease in GSH. The decreased ascorbic acid levels in our study were concomitant with
that of Ramanathan et al (2002, 2003a, b) who have reported reduced ascorbic acid levels in arsenic intoxicated rats. Earlier work done in our laboratory using ascorbic acid in mice ovary has documented comparable results with that of the present data (Chinoy et al., 2004). Alteration in ascorbic acid metabolism has also been reported in several organs of arsenic treated rats and mice (Chinoy, 2002; Rao and Avani, 2006).

Inorganic arsenic increases the rate of formation of active oxygen species including superoxide anion radical (O$_2^-$), hydroxyl (OH-) radical and peroxyl (ROO-) radicals through a chain reactions (Yamanaka et al., 1989a, b, 1990; Pi et al., 2002). Oxidative stress, which is based on activated molecular species of oxygen, is a complex process that can result in the peroxidative damage of the major cellular components including amino acids, carbohydrates, lipids, proteins, and nucleic acids (Sies H, 1985; Belloma and Orrenius, 1985). Arsenic exposure can enhance the production of ROS (Chen et al., 1998; Tseng, 2004), interfere with the activity of key antioxidant enzyme such as glutathione reductase, glutathione S-transferase, glutathione peroxidase and glucose-6-phosphate dehydrogenase (Maiti and Chatterjee, 2000; Santra et al., 2000), and induce lipid peroxidation (Santra et al., 2000). Acute and chronic intake of arsenic increased lipid peroxides in blood, liver, kidney and other organs of rats (Ramos et al., 1995; Flora, 1999). In our study, the ROS generated by arsenic react with polyunsaturated fatty acid and cause peroxidative changes that result in enhanced lipid peroxidation, thus corroborating well with the earlier speculation that arsenic induces oxygen free radicals or promotes formation of lipid peroxides (Borcea et al., 1999; Ramos et al., 1995).
The enzymatic antioxidants, SOD, CAT and GPx counteract the free radical and reduce the oxidative stress. SOD accelerates the conversion of superoxide radical to hydrogen peroxide while CAT or GPx converts hydrogen peroxide to water. An increase in LPO level was presumably associated with increased free radicals, confirming the fact that the free radicals reduced the antioxidant status in endocrine organs of arsenic treated mice. The observed alterations might be attributed to the utilization of these antioxidants to alleviate free radical induced oxidative stress. Glutathione is a tripeptidyl molecule and is present either the reduced (GSH) or the oxidized state (GSSG) by forming a disulfide bond between two molecules. It has pleiotropic roles which include the maintenance of cells in a reduced state and the formation of conjugates with some harmful endogenous and xenobiotic compounds. In our study, glutathione levels were found to be significantly decreased. This reduction is suggested to be due to the consumption of glutathione while protecting against the arsenic induced oxidative stress. Cellular toxicity of arsenic was found to be inversely related to intracellular GSH levels and thus may be enhanced by GSH depletion (Ochi et al., 1996; Pi et al., 2000).

Sulphydryl groups are prone to be damaged by free radicals resulting in their oxidation (Halliwell and Gutteridge, 1985). A steep decline in the levels of total thiol (sulphydryl) groups was noted in pancreas after arsenic exposure. A number of sulfhydryl group containing proteins and enzyme systems have been found to be altered by exposure to arsenic (Robert and Judd, 1986; Roy and Saha, 2002). The present study also discovered that exposure of arsenic possibly induces diabetes mellitus in female mice,
marked by change of normal blood glucose level (Biswas et al., 2000). Arsenite has high affinity for sulfhydryl groups and thus can form covalent bonds with the disulfide bridge in the molecules of insulin, insulin receptors, glucose transporters (GLUTs), and enzymes involved in glucose metabolism. As a result, the normal functions of these molecules could be hampered.

A relationship in between arsenic induced oxidative stress in pancreas (Mukherjee et al., 2006) and development of diabetes mellitus has also been proposed by many researchers (Borcea et al., 1999). Acinar cells produce large amounts of ROS at early stage of pancreatitis (Urunuela et al., 2002). Highly reactive ROS directly attacks lipids, proteins in the biological membranes and cause their dysfunction (Yamamato et al., 1985). When the production of ROS is increased in pancreatitis, the capacity of intrinsic defense mechanism leads to alteration in cytoskeleton of acinar cells and damage of cell membranes (Dabrowski et al., 1999). Degradation of polyunsaturated fatty acids in cell membranes by ROS results in the destruction of membranes and formation of MDA, which is an indicator of ROS generation (Dabrowski et al., 1988). Disruption of cytoskeleton leads to disturbance of intracellular transport of digestive enzymes and damage in acinar cells (Jungermann et al., 1995). Large amounts of ROS and activated pancreatic enzymes leaked from the broken cells injure capillary endothelium. In the present study, a markedly increased amylase and lipase activity in pancreas was the indication of extensive tissue and cellular damage in arsenic treated animals. In support to our data studies by Hotaling, (1998) reported that arsenic administration elevated the serum amylase due to impairment of acinar tissues. In histological findings, pancreatic tissue
displayed sign of inflammation, tissue damage, vacuolization, damage of acini, rupture of islet cell, shrinkage and reduced number of islet cell. Thus, histopathological changes coincide with the altered biochemical studies, which indicate extent of toxicity developed by arsenic on pancreas.

In present study, arsenic induced oxidative stress observed in thyroid gland of treated animals as revealed by increase in lipid peroxidation and decrease in antioxidative indices like GSH, SOD, GPx and CAT. In histological analysis, thyroid tissue showed several microfollicles, interfollicular edema, reduced colloid content of the follicles, and hypertrophy of glandular epithelial cells as a result of oxidative stress exerted by arsenic as described earlier. This result was also supported by Glattre et al (1995) and Biswas et al (2000) which indicate extent of toxicity developed by arsenic on rat thyroid.

Arsenic accumulation in the various organs further supported arsenic exerted toxic effects by affecting its structural, biochemical and functional aspects, in support of earlier published data (Rao and Avani, 2004; Modi et al., 2007). The above data clearly elucidates alterations in endocrine organs structure which would influence their functions.

ROLE OF MELATONIN

Antioxidants neutralize and/or metabolically remove reactive species from cells before they carry out their destructive activities. While the antioxidative functions of most molecules are limited by their specific intercellular distribution, antioxidative actions of melatonin include the protection of lipids in cell membrane, proteins in cytosol and DNA in nuclei. Melatonin is readily absorbed when it is administrated via any route; it crosses
all morphophysiological barriers with ease, it seems to enter all parts of every cell where it prevents oxidative damage and preserves mitochondrial functions (Reiter et al., 1999; Acuña-Castroviejo et al., 2001). Considering this, the aim of this work was to investigate the prophylactic role of melatonin in reducing oxidative stress and histological changes in the endocrine organs of arsenic treated mice.

Co-administration of melatonin with arsenic could impart a protective effect against arsenic-induced toxicity on the metabolic, steroidogenic, antioxidative, serum parameters and arsenic retention in the ovary, adrenal, pancreas and thyroid gland of mice. MLT supplementation also regained the weight loss of body and organs induced by arsenic. Similarly, ingestion of MLT with arsenic showed improvement in the histology of ovary with curing in the stromal tissue and the follicles found to be normal with no vacuolization. A pronounced improvement in the corticomedullar demarcation of adrenal glad was observed. A comprehensive recovery in the morphology and population of cells in pancreas were observed. Treatment of MLT appeared normal follicles, colloid and interfollicular region in the thyroid gland (Kundurovic and Sofic, 2006). Thus, hispathological examination of the mentioned endocrine organs did not show any changes of pathologic significance after being treated with melatonin, which recommended the possibility of treating arsenic intoxication with MLT. Collectively, these findings may together explain why melatonin is superior to other antioxidants such as vitamin C, vitamin E, N-acetyl cysteine and glutathione as verified by studies (Khaldy et al., 2000; Yilmaz et al., 2002; Tan et al., 2003).
Melatonin is one of the most effective oxygen free radical scavengers. The protective effect of melatonin was shown in a very wide variety of studies on xenobiotics (Daniels et al., 1995; Melchiorri et al., 1996; Giusti et al., 1996; Dzięgiel et al., 1997). Also, the protective effects of melatonin were evident in many different organs (brain, kidneys, bone marrow, liver), as illustrated in earlier reports (Montilla et al., 1996; 1997; Rapozzi et al., 1998, 1999). Numerous reports have documented protective actions of melatonin in various models of oxidative stress (El-Sokkary et al., 1999, 2000; Lee et al., 2002; Parlakpinar et al., 2002). This is due to its high efficacy as a free radical scavenger and indirect antioxidant (Reiter et al., 1999,2000; Tan et al., 2000, 2002; Allegra et al., 2003). Additionally, this indole detoxifies other reactive oxygen and nitrogen species including single oxygen [1O2] (Zang et al., 1998), nitric oxide [NO] (Turjanski et al., 2001), the peroxinitrite anion (ONOO⁻) as well as its metabolite and hydrogen peroxide (Blanchard et al., 2000). Finally, melatonin stimulates the activities of antioxidative enzymes that metabolize reactive species (Reiter et al., 2000) and maintains cell membrane fluidity at an optimal level (Garcia et al., 1998).

Melatonin influenced cholesterol metabolism (Tamura et al., 2008), and found to enhance the steroidogenesis (Adriaens et al., 2006; Gangadharan, 2008), which was drastically reduced in the arsenic treated animals, suggested that arsenic induced ovarian and adrenal damage was reversed by MLT ((Pertsov, 2006). In support to our data, protection against altered protein levels has also been reported in melatonin treated rats (Gangadharan, 2008). Arsenic induced lipid peroxidation in all the mentioned endocrine organs. The gradual reduction in LPO in these organs after MLT
co-treatment reveals that MLT provides substantial protection against the oxidative destruction of lipids in arsenic treated animals subjected to stress, which is in corroboration with earlier studies (Pal and Chatterjee, 2006). Melatonin may protect against extensive oxidative damage in the case of a harmful action of some external factors on the thyroid during physiological and pathological processes. Karbownik and Lewiński (2003) reported indoleamine protection against lipid peroxidation in the porcine thyroid. Moreover, Kanno et al (2006) also reported MLT may act not only to prevent toxicity but also as a detoxification medicine. Administration of arsenic to experimental animals might lower the activity of superoxide dismutase, catalase and glutathione peroxidase. Glutathione peroxidase is one of the key enzymes of antioxidant defense and its activity in cells of various organs is stimulated by melatonin. Sanfey et al (1984), Dąbrowski et al (1999), and Pal and Chatterjee (2006) also reported that besides melatonin’s ability to scavenge of ROS, this substance has been demonstrated to activate the antioxidative enzymes such as catalase (CAT) and glutathione peroxidase (GPx). Melatonin supplementation led to an increase in SOD activity in accordance with the findings of Antolin et al (1996) who reported that administration induces an increase in the mRNA levels of SOD.

Nevertheless, in spite of the antioxidant property demonstrated by melatonin, the studies involving its use for the treatment of oxidative stress induced damage in pancreas is limited (Anderson and Sandler, 2001; Vural et al., 2001). In this sense, the study conducted by Jaworek et al (2003) has shown that the administration of melatonin reduces lipid peroxidation, pancreatic edema, and improves amylase concentration in serum. In
concordance with others (Muñoz-Casares et al., 2006; Jaworek et al., 2004; Eşrefoğlu et al., 2006), we have also shown a significant reduction in amylase and lipase concentration in serum after melatonin treatment. It was observed that melatonin prevented the animals from alloxan-induced diabetes by scavenging of hydroxyl radical produced in pancreatic β-cells (Ebelt et al., 2000; Yavuz et al., 2003).

In the present study, accumulations of arsenic in endocrine organs were also lowered by MLT, but the actual mechanism behind this action remains unclear. Histopathological examination of endocrine organs also showed improvement in the structural alterations after melatonin administration. Probably, such protection against the development of histopathological changes might be attributed partly to the antitoxic action of GSH restored by melatonin, because GSH has multiple functions in detoxification, and its decrease has been associated with an increased risk of chemical toxicity.

Free radical scavengers and antioxidants neutralize and metabolically remove reactive species from cells before they carry out their destructive activities. Several compounds have been tested against arsenic toxicity. Selenium can directly counteract arsenic toxicity (Davis et al., 2000). Dimercaptosuccinic acid (DMSA) has been found globally ineffective in treating chronic arsenic toxicity (Guha-Mazumdar et al., 1998). However, vitamin E offered protection against ovarian toxicity of sodium arsenite (Chattopadhyay et al., 2002). Some investigators have also showed the antioxidants ascorbic acid and alpha-tocopherol were able to modulate arsenic toxicity (Ramanathan et al., 2003a). Our study followed the same purpose of screening two different compounds (Andrographis paniculata and
melatonin) with antioxidant capacity in order to identify which of them displays higher protection against arsenic toxicity. *Andrographis paniculata*, is one of the main ingredients of several Ayurvedic and herbal formulation and has been proposed to be a potential therapeutic agent in the treatment of different pathologies in which oxidative stress is involved. It possesses anti-diabetic, antipyretic, hepatoprotective, anti-inflammatory and antioxidant properties. Melatonin is a ubiquitously acting molecule with several functions. In recent years, melatonin’s ability to influence immune function and its antioxidative properties has been uncovered. In the present study, treatment with antioxidant agents AP and MLT, were capable of limiting endocrine organs damage produced during arsenic treatment *via* restoring tissue antioxidant defense mechanism.